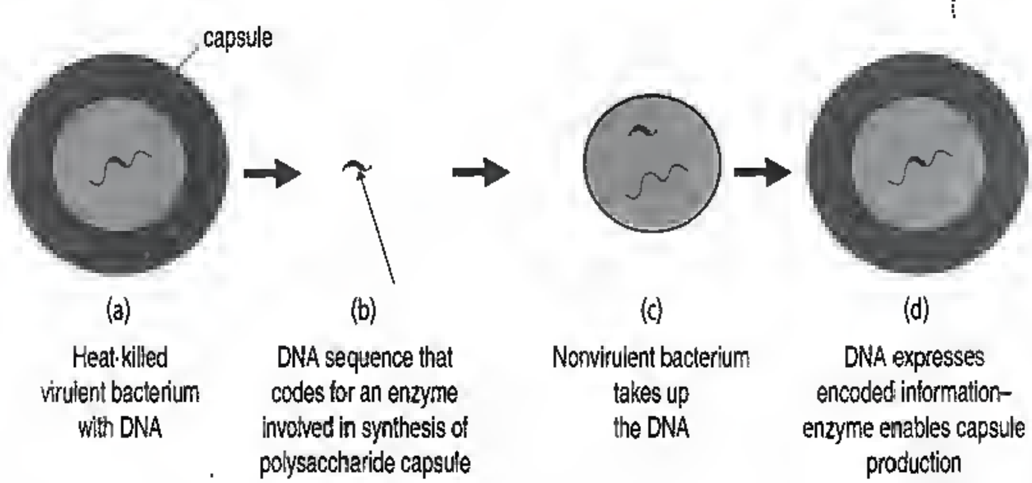
LEARNING 7 EXPERIENCE

PROTEIN, THE WONDER INGREDIENT

PROLOGUE The experiments of Griffith and of Avery and his colleagues demonstrated that DNA contained information enabling the bacteria *Diplococcus pneumoniae* to produce a polysaccharide capsule, and that this capsule allowed the bacteria to grow and flourish in the host, which would exhibit the symptoms of pneumonia.

What is the relationship between DNA and the ability of *D. pneumoniae* to synthesize a protective capsule? In the last learning experience, you explored the processes involved in the translation of information from DNA into protein. The difference between the nonvirulent and virulent strains was actually a difference in just one enzyme—the virulent strain contained a specific enzyme that enabled it to synthesize a polysaccharide capsule. The information that was transferred, transforming a nonvirulent strain to a virulent strain, was in the DNA code. Because it enabled the nonvirulent strain to produce the enzyme that could then facilitate the synthesis of the polysaccharide capsule, the presence of this DNA sequence determined that this strain would now be virulent (see Figure 7.1).

Figure 7.1
Transforming factor makes a new enzyme.



Proteins are essential components of all living organisms and can carry out enormously diverse functions. How do proteins do what they do? What makes them uniquely able to carry out a wide range of functions, thereby directing a great variety of chemical activities? In this learning experience, you will explore the function of these wondrous molecules and examine how their structure permits them to carry out these functions.

READING

Protein, Protein, Everywhere

Living organisms contain an immense assortment of biomolecules. The simplest life forms, bacteria, contain about 5,000 different biomolecules, including 3,000 different kinds of proteins. In humans, the numbers and the variation are even greater. It is this diversity of protein biomolecules that enables cells to carry out the myriad of activities involved in life processes. Proteins are a good example of the simplicity within diversity; they are all chains of amino acids. It is the number and order of the amino acid subunits that result in the diversity of proteins; this diversity then determines differences in characteristics of cells, and therefore of whole organisms.

Each type of protein is superbly efficient at one task. How many proteins exist in the human body? No one knows, but a hundred thousand is not a bad guess, and though none of these protein molecules is identical to any found in bacteria, some of their functions are quite similar.

What are these proteins? Why are there so many different kinds? What do they do and how do they do it? Actually, you are probably familiar with proteins—they are the "wonder ingredients" in products you use every day, such as the laundry detergents that claim they can get out your toughest stains, and shampoos that are supposed to make your hair look terrific.

You may be most familiar with the proteins in the foods you eat. Proteins are an essential part of your daily diet. Plants can make their proteins from carbon dioxide, water, nitrates, sulfates, and phosphates; animals, on the other hand, can synthesize only a limited number of proteins and are mainly dependent on plants or other animals as dietary sources of protein. Protein intake is required regularly in animals because their bodies have little stored protein. If excess protein is taken in, more than the body can use, it is stored as fat.

Proteins are essential to the structure and function of all living organisms. Next to water, protein is the most abundant substance in cells. Proteins make up half of the solid matter in cells (water makes up 70% of the cell) and perform a wide variety of tasks in organisms. These tasks can be classified by biological function.

The largest class of proteins consists of the *enzymes*, protein mole-

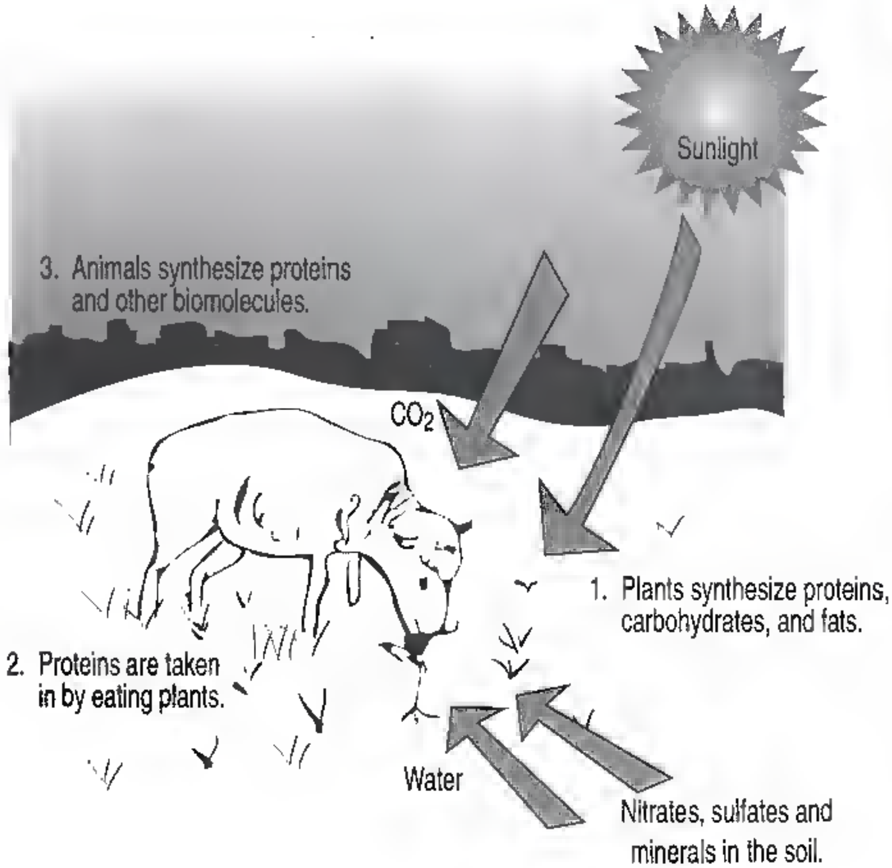


Figure 7.2
Protein flow in nature

cules that facilitate and enhance the rate of chemical reactions in the cell. That is, enzymes assist in chemical reactions so that the reactions proceed with speed and efficiency. Without enzymes, the metabolic reactions of the cell would still take place but far too slowly to sustain life; it would take you about 50 years to digest your lunch. Reactions such as those involved in the breakdown of food, the synthesis of cell components, or the transport and storage of energy that otherwise might take days or weeks or years to happen, occur in milliseconds in the cell because of the action of enzymes. Examples of enzymes include: lactase which breaks down the milk sugar lactose into glucose and galactose; catalase which degrades hydrogen peroxide to oxygen and water; RNA polymerase which is responsible for transcribing the information in DNA into mRNA; and dipbosphoribulose carboxylase which fixes carbon dioxide (CO_2) into sugar in photosynthetic organisms. Note that the suffix -ase is usually found in an enzyme's name.

The second major class of proteins makes up the structural components of cells and tissues. Collagen, the major structural protein in connective tissue and in bone, is also part of the "glue" that binds a group of cells together to form a tissue. Another structural protein, keratin, gives strength to skin, hair, nails, horns, and feathers.

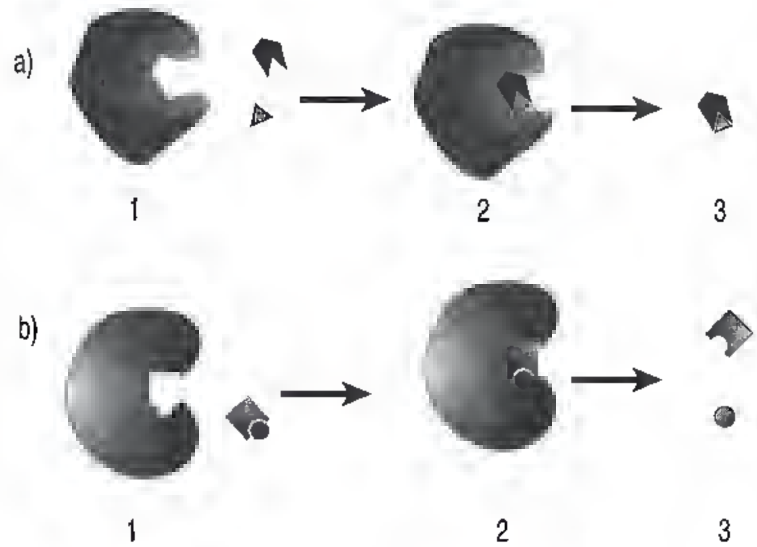
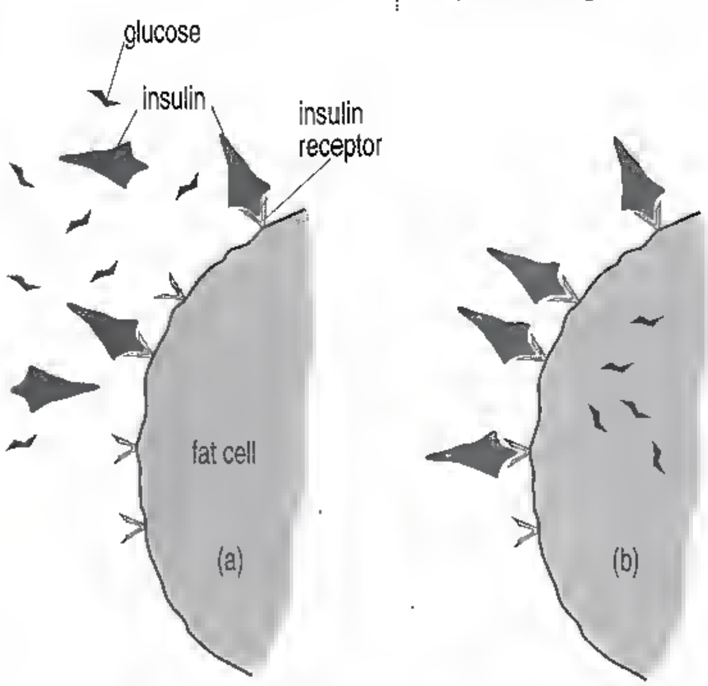


Figure 7.3
Enzymes facilitate anabolism (a) by putting molecules together and (b) facilitate catabolism by taking molecules apart.

In addition, proteins carry out other essential types of functions. Actin and myosin are the two major proteins that enable muscles to contract. Some proteins have a transport function: hemoglobin in blood carries oxygen around the body; myoglobin transports oxygen through muscle tissue; and serum albumin transports fatty acids through the blood to various organs. Hormones such as insulin (which regulates blood sugar) and somatotropin (a growth hormone) are proteins. Proteins found in the immune system, such as antibodies, protect us from infection. In contrast, toxins—proteins made by organisms including bacteria, plants, and snakes—may do us great harm.

Figure 7.4
(a) Insulin binds to a protein receptor on fat cells (b) causing the cells to take up glucose from the blood.



Many proteins act by binding to other proteins and triggering a specific cellular response. For example, the hormone insulin controls the level of glucose in the blood. Insulin acts by binding to specific proteins on the surface of muscle, liver, and fat cells. This binding causes a change in their cell membranes that results in their taking up glucose from the blood, thus reducing the concentration of blood glucose (see Figure 7.4).

Proteins also can serve as a source of energy for cells. If the diet does not supply enough fat and carbohydrate, which are the primary sources of energy in the cell, stored fat is broken down for energy. But if stored fat is used up, as in starvation or extreme dieting,

then proteins can be used for energy, instead, even at the expense of building new cells and maintaining tissue structure.

How do proteins carry out all of these essential functions? How does the structure of a protein relate to its function? The shape of an individual protein is very important in its function. All proteins have a linear sequence of amino acids, but the molecules are folded into complex three-dimensional structures. The shapes of these structures are determined by which amino acids make up the proteins and the sequence in which they occur. Just looking at the sequence of amino acids in a protein cannot tell us how it folds; it cannot tell us how growth hormone makes us taller or how a bacterial toxin can kill us. But the information in that sequence plays an essential role in determining if and how these interactions take place. Exactly how the sequence of amino acids determines a protein's function is not completely understood, but some of the pieces of the puzzle have been determined, as you will see in your investigation.

LIVER AND LET LIVER

ACTIVITY

INTRODUCTION Liver may not be one of your favorite foods, but the liver in your own body should be one of your favorite organs. It plays the life-saving function of detoxification. Every day your body takes in toxic substances from the air and the food you eat. In addition, some of the byproducts of your cells' metabolic activities are toxic to your system. The job of the liver is to neutralize and dispose of these toxic substances—a sort of hazardous waste disposal. Without a liver, hazardous products would soon build up, inhibit vital metabolic processes, and result in death.

One of these toxic metabolic byproducts in animal tissues is a molecule called hydrogen peroxide. You may be familiar with hydrogen peroxide as a substance which can be used for bleaching hair or for disinfecting wounds to prevent infection. Hydrogen peroxide (H_2O_2) is toxic to living things because it produces something called a superoxide radical, O_2^-, from oxygen. This radical can destroy certain biomolecules such as proteins. Therefore, it is essential that organisms be able to dispose of hydrogen peroxide. Catalase is an enzyme present in liver cells (and other plant and animal cells) that neutralizes this hazardous waste product by breaking it down into harmless molecules. In the following investigation, you will examine the activity of catalase.

MATERIALS NEEDED

For each pair of students:

- 2 pairs of safety goggles
- 2 small pieces of liver (one cooked and one raw)
- 2 flasks (125- to 250-mL) or test tubes (13 x 100 mm)
- 2 balloons
- 50–100 mL hydrogen peroxide

SAFETY NOTE: *Always wear safety goggles when conducting experiments.*

CAUTION: *Avoid getting the hydrogen peroxide in your eyes, and don't heat it. It should be rinsed off the skin immediately if contact occurs.*

PROCEDURE

1. Place raw liver in one flask or test tube and cooked liver in another flask.
2. Pour enough hydrogen peroxide into each flask to cover each piece of liver.
3. If possible, cover the top of each flask or test tube with a balloon to capture the gas which is being formed.
4. Observe the reaction for 5–10 minutes. Write your observations in your notebook and write responses to the following Analysis questions.

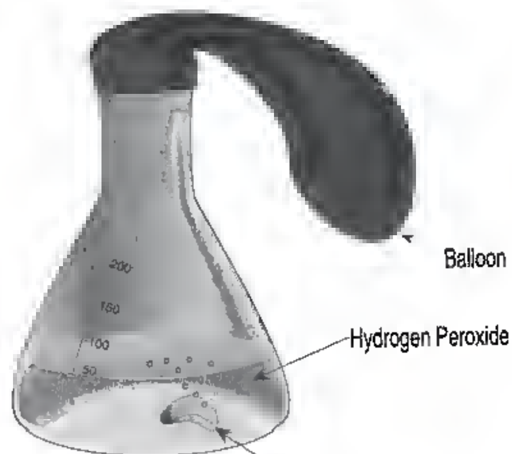


Figure 7.5
One flask for "Liver and Let Liver" investigation

ANALYSIS

1. What happened when hydrogen peroxide was added to the raw liver?
2. The chemical formula for hydrogen peroxide is H_2O_2. What gas do you think was produced? How might you identify the gas?
3. What else might have been produced?
4. Write a chemical equation which describes what happened.
5. How was the reaction in the cooked liver different from the reaction in the raw liver?
6. Based on your knowledge from the readings, what was the cause of the difference in these two reactions?

GETTING INTO SHAPE

ACTIVITY

INTRODUCTION The first step in most protein reactions is for one protein to bind to another molecule. In the case of enzymes, this binding step brings the second molecule, which could be another protein, a sugar, a lipid, or a nucleic acid, into position so that the enzyme can act on it. Transport proteins, such as hemoglobin and certain membrane proteins, bind molecules in order to move them from place to place. Structural proteins, sucb as collagen, may bind to one another to give shape and strength to the structure.

These binding interactions occur at specific sites on the protein molecules (*binding sites*). The binding site on a protein is defined by the shape of the protein. The folding of the protein results in a groove, cleft, or pocket on its surface into which the second molecule fits, much like a key in a lock. If the shape of this binding site is altered, the ability of the protein to interact with the second molecule may be lost, rendering the protein unable to carry out its function. Once the second molecule binds to the protein, that enzyme can then do its job, whether that is an enzymatic reaction, a transport function, a structural function, or something else.

The principles of *protein folding* are one of the important, unsolved mysteries of science. It is known so far that the amino acid composition and the order in which these amino acids occur in the protein play a central role in determining how a protein will fold. Amino acids have a characteristic structure and differ from one another in their side groups, which may have a positive, negative, or neutral charge (see Figure 7.6). The positive and negative charges on the amino acids appear to be important in folding. Amino acids also demonstrate *hydrophobic* (water-fearing) and *hydrophilic* (water-loving) properties seen in lipids. These properties are also important in folding, as the water-fearing parts tend to move to the interior of the molecule, away from the water. Another factor involved in folding is the ability of amino acids to form chemical bridges or bonds between eacb other.

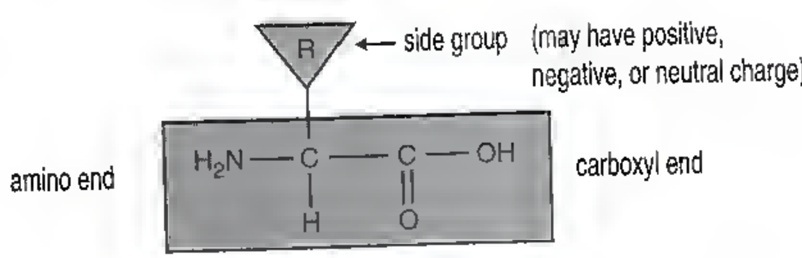


Figure 7.6
Structure of an amino acid

In this activity, you will build a model that demonstrates the first step in protein function, the binding of a protein to another molecule.

MATERIALS NEEDED

For each pair of students:

- 3–4 extra-long pipe cleaners
- 1 set of 50 colored beads that can be threaded on the pipe cleaners
  - 10 red beads, representing positively charged amino acids
  - 10 green beads, representing negatively charged amino acids
  - 30 beads of a third color, representing neutral amino acids
- 1 container to hold beads
- 1 small geometrically shaped object such as a ball, a triangular block, or a cube
- cellophane tape

PROCEDURE

NOTE: Many forces are involved in determining protein folding. For the purposes of this activity, you will consider only the attractive forces of amino acids (positive, negative, and neutral charges) in determining how a protein folds.

1. Join 3–4 long pipe cleaners together securely, end to end. Be sure that you can't tug them apart.
2. Thread the beads along the pipe cleaners in random order. The protein molecule model must be built of pipe cleaners (representing peptide bonds) and no fewer than 25 beads (representing amino acids).
3. Twist your protein molecule model around the three-dimensional object (the other molecule). In twisting your molecule two features are important:
   - The interaction of the protein molecule and the other molecule must show "specificity"—the binding molecule and the binding site must mirror one another; that is, they should match in shape and fit together in a lock-and-key fashion. A pocket or groove along the edge of the pipe cleaner should form, molding around the small object (such as a ball or triangular block) which represents the other molecule involved in the interaction. The goal is to make a space in the protein molecule into which the other molecule fits (relatively) precisely.
   - The protein molecule must be folded according to the principle of mutual attraction. Positively charged amino acids (red) will attract negatively charged amino acids (green). Neutral ones will have no effect on folding. In forming this pocket be sure to have two or three locations where red and green beads come together, as they might in a protein in which the folding is dependent in part on interactions between positively and negatively charged amino acids. This may require some squeezing or sliding of beads. You may wish to stabilize this "interaction" with pieces of cellophane tape.

4. In your notebook, draw a diagram of your model, indicating in words or drawings:
   - where the binding site is on your protein;
   - how the interacting molecule fits or binds at this site;
   - where the important interactions between the protein and the other molecule occur; and
   - how the structure of the protein defines the binding site.
5. Find a red bead that is important in forming the shape of the binding site, and substitute one green bead for it; also substitute a "neutral" bead for one red or green bead, which is important in forming the binding site. What happens to the shape of your protein? What happens to the shape of your binding site? Write responses in your notebook.
6. Remove (delete) a section of your protein. (You may cut the protein molecule, remove the cut piece, and rejoin cut ends if you wish.) Does this change the protein shape or binding site?
7. Write responses to the following Analysis questions and be prepared to discuss your model in class.

ANALYSIS

1. Describe the factors that determine the shape of your protein model.
2. How have changes in the shape of your protein influenced the protein's ability to bind to your other "molecule"?
3. How does altering the shape of your protein change its function?
4. Describe the steps involved when the enzyme lactase breaks down lactose sugar into glucose and galactose. What would happen if the enzyme binding site were altered? What would be the consequences for the organism if this enzyme were altered?
5. Some molecules have more than one binding site. For example, RNA polymerase, the enzyme that copies the encoded message in the DNA into mRNA, has binding sites both for the DNA molecule and for RNA nucleotides. What would happen to the ability of this enzyme to carry out its function if one of the binding sites was altered or lost? What would be the consequences for the organism?
6. Based on what you know about the transfer of information from DNA to protein, how might a change in the amino acid sequence of your protein occur?

EXTENDING IDEAS

- Investigate diseases that are the result of changes in the sequence of a protein. Examples of this include sickle cell anemia, hemophilia, cystic fibrosis, Huntington's chorea, or lactose intolerance. Determine the symptoms of the disease, what protein is affected, what the function of that protein is (if it is known), what change has occurred to the protein, and how this is reflected in the DNA sequence (if it is known).
- Research how carbon monoxide poisoning occurs. What effect does carbon monoxide have on animals? How does it bring this effect about? Describe how this is an example of the principle, "another key that fits the lock." What does carbon monoxide do to the "lock?"
- A permanent wave is an example of the altering, or denaturing of proteins in hair. Explore the chemistry of a "perm."

ON THE JOB

PROTEIN CHEMIST Did you know that no two proteins are alike? Protein chemists study the physical and chemical structures of proteins. Protein chemistry is one field of biochemistry, the study of the chemical reactions in living organisms and the effect of chemicals on life processes. Some protein chemists are involved in basic research which might involve isolating proteins by charge, mass, or shape, looking at their chemical structure and how each protein folds, or using the technique of crystallography to identify crystal structure which provides clues to the protein's function. Protein chemists involved in applied research might be involved in developing synthetic forms of proteins. Protein chemists work with chemical technicians in either a basic research laboratory or an applied research laboratory. Chemical technicians would look at the chemical content of a product, its purity, strength or stability or how to create new chemical products. This might include taking measurements, making calculations or collecting and analyzing data. A position as a chemical technician is available with a high school diploma or a two year college degree, as a laboratory assistant with a four year college degree. With a master's or doctoral degree chemists might be employed in supervisory positions in industry or pursue independent research in a university. Classes such as biology, chemistry, physics, algebra, geometry, foreign languages, English and computer science are recommended.

SCIENTIFIC ILLUSTRATOR Are you intrigued by science, medicine and technology, but find your true passion to be drawing and illustration? Scientific illustrators combine their talent and technical ability in illustration with their scientific background to produce charts, graphs, illustrations or cover art for scientific articles and books. A scientific illustrator might be employed by a company or work as a freelance artist. Possible employers and clients are scientific laboratories in universities or in businesses, and publishers of magazines (both professional journals and those written for the general public), newspapers, and books. The illustrator's grasp of the subject matter is important in conferring with the doctors or scientists requesting the art work. Illustrations are created using computer graphics as well as by hand drawing. Some types of illustrations (such as medical illustrations) are better drawn by hand than by using the computer, because the artist is better able to portray the level of detail and complexity. Scientific illustration is a specialty of illustration and drawing because of its focus. Medical illustration is a specialty of scientific illustration. Medical illustrators enter special degree programs. In addition to taking computer graphics and drawing courses, they complete the first year of medical school, which includes anatomy classes and cadaver dissectionsp. Scientific illustrators usually have at least a four year college degree. Classes such as biology, chemistry, physics and other sciences, drawing, illustration, computer graphics, business courses, and English are recommended.